Scientific Reports - Supporting Information

Article title: Evolution of sex determination and heterogamety changes in section *Otites* of the genus *Silene*

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Methods S1: Segregation patterns of Y-linked SNP (male heterogamety) and corresponding filtering rules used in LINKYX.

Pattern A: P: $X^1 X^1$ x $X^1 \underline{Y^2}$

(Homozygous mother)

$\downarrow$

F$_1$: $X^1 X^1$ x $X^1 \underline{Y^2}$

Pattern B: P: $X^1 X^3$ x $X^1 \underline{Y^2}$

$\downarrow$

F$_1$: $X^1 X^1$ x $X^1 \underline{Y^2}$

$X^3 X^1$ x $X^3 \underline{Y^2}$
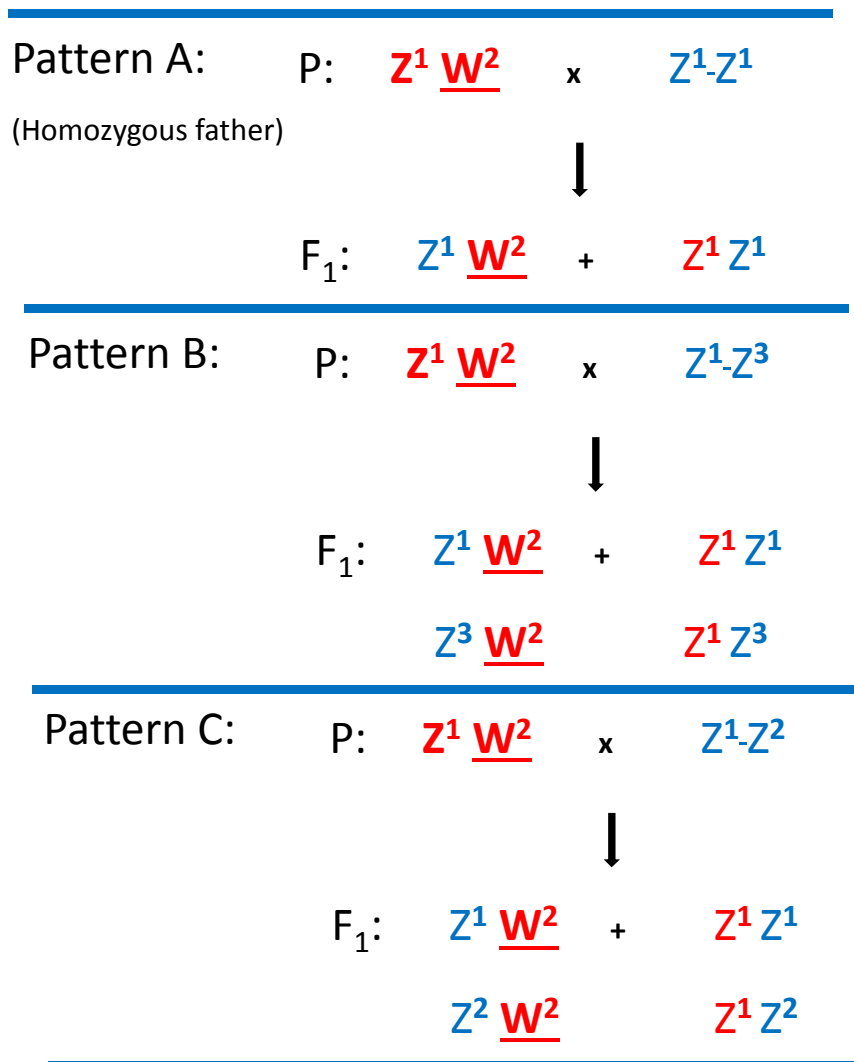
Pattern C: P: $X^1 X^2$ x $X^1 \underline{Y^2}$

$\downarrow$

F$_1$: $X^1 X^1$ x $X^1 \underline{Y^2}$

$X^2 X^1$ x $X^2 \underline{Y^2}$

Filtering rules for identification of Y-linked genes following segregation pattern A:

1) **Mother must be homozygous ($X^1 X^1$)** - Tolerance according to depth of coverage:
   0-4 reads: 0,
   5-24 reads: 1,
   >24 reads: <=4 %
2) **Sons must be same as father ($X^1 Y^2$)**
3) **Father must differ from mother (presence of extra allele X^2).**
4) **Sons must have mother and father alleles ($X^1 Y^2$)**
5) **No "recombinant" individuals are allowed** - Tolerance according to depth of coverage:
   0-4 reads: 0,
   5-24 reads: 1,
   >24 reads: <=4 %.
6) **Sufficient coverage.** Average depth per nucleotide must be at least 0.75 (=in contig of length 100, there must be at least 75 nucleotides present/mapped).

Methods S2: Segregation patterns of W-linked SNP (female heterogamety) and corresponding filtering rules used in LINKYX.

Pattern A: P: $Z^1 \underline{W^2}$ x $Z^1\text{-}Z^1$

(Homozygous father)

F_1: $Z^1 \underline{W^2}$ + $Z^1 Z^1$

Pattern B: P: $Z^1 \underline{W^2}$ x $Z^1\text{-}Z^3$

F_1: $Z^1 \underline{W^2}$ + $Z^1 Z^1$

$Z^3 \underline{W^2}$ $Z^1 Z^3$

Pattern C: P: $Z^1 \underline{W^2}$ x $Z^1\text{-}Z^2$

F_1: $Z^1 \underline{W^2}$ + $Z^1 Z^1$

$Z^2 \underline{W^2}$ $Z^1 Z^2$

Filtering rules for identification of W-linked genes following segregation pattern A:

1) **Father must be homozygous (Z^1Z^1)** - Tolerance according to depth of coverage:
   0-4 reads: 0,
   5-24 reads: 1,
   >24 reads: <=4 %
2) **Daugthers must be same as mother (Z^1W^2)**
3) **Mother must differ from father (presence of extra allele W^2).**
4) **Daugthers must have mother and father alleles (Z^1W^2)**
5) **No "recombinant" individuals are allowed** - Tolerance according to depth of coverage:
   0-4 reads: 0,
   5-24 reads: 1,
    >24 reads: <=4 %**.**
6) **Sufficent coverage.** Average depth per nucleotide must be at least 0.75 (=in contig of length 100, there must be at least 75 nucleotides present/mapped).

Methods S3: Segregation patterns of Z-linked SNP (female heterogamety) and corresponding filtering rules used in LINKYX.

Pattern A: P: $\underline{Z^2}\,W^1$ x $Z^1\text{-}Z^1$

(Homozygous father)

F_1: $Z^1\,W^1$ + $\underline{Z^2}\,Z^1$

Pattern B: P: $\underline{Z^2}\,W^1$ x $Z^1\text{-}Z^3$

F_1: $Z^1\,W^1$ + $\underline{Z^2}\,Z^1$

$Z^3\,W^1$ $\underline{Z^2}\,Z^3$

Pattern B: P: $\underline{Z^2}\,W^1$ x $Z^1\text{-}Z^2$

F_1: $Z^1\,W^1$ + $\underline{Z^2}\,Z^1$

$Z^2\,W^1$ $\underline{Z^2}\,Z^2$

Filtering rules for identification of Z-linked genes following segregation pattern A:

1) **Father must be homozygous (Z^1Z^1)** - Tolerance according to depth of coverage:
   0-4 reads: 0,
   5-24 reads: 1,
   >24 reads: <=4 %
2) **Sons must be same as mother (Z^2Z^1)**
3) **Mother must differ from father (presence of extra allele Z^2).**
4) **Sons must have mother and father alleles (Z^1Z^2)**
5) **No "recombinant" individuals are allowed** - Tolerance according to depth of coverage:
   0-4 reads: 0,
   5-24 reads: 1,
   >24 reads: <=4 %.
6) **Sufficient coverage.** Average depth per nucleotide must be at least 0.75 (=in contig of length 100, there must be at least 75 nucleotides present/mapped).

Methods S4: Filtering rules for the identification of the contigs with sex specific transcription.

I. Filtering rules for identification of male specific genes

1) Minimal and maximal number of reads: Each male must have at least 15 reads mapped, each female can have at most 3 reads mapped. Reference is based on reads from all males. Before applying filtering rules, read numbers are normalized to the number of mother reads.

2) Fraction of female and male reads: female reads/(female reads + male reads) < 0.03.

3) Male reads must be more abundant than female reads.

4) mother reads < father reads;

5) mother reads < son reads;

6) daughter reads < father reads;

7) daughter reads < son reads

II. Filtering rules for identification of female specific genes

1) Minimal and maximal number of reads: Each female must have at least 15 reads mapped, each male can have at most 3 reads mapped. Reference is based on reads from all females. Before applying filtering rules, read numbers are normalized to the number of father reads.

2) Fraction of male and female reads: male reads/(female reads + male reads) < 0.03.

3) Female reads must be more abundant than male reads.

4) father reads < mother reads;

5) father reads < daughter reads;

6) son reads < mother reads;

7) son reads < daughter reads

Methods S5: BAC library construction.

Briefly, 25g of *S. otites* fresh leaf tissue was formaldehyde-fixed and homogenized. The crude suspension of intact nuclei was purified by flow-sorting (FACSAria, Becton Dickinson) and embedded into agarose plugs, each averaging 200,000 nuclei. 11 plugs were partially digested with *Hin*dIII enzyme. The digested DNA was separated by pulsed field gel electrophoresis in two size-selection steps, yielding 100-300 kb fragments which were then isolated from the gel and ligated into a dephosphorylated vector pAGIBAC5. The recombinant BACs were transformed into the *E. coli* strain MegaX DH10B T1 (Invitrogen) by electroporation. The library consists of 73,728 clones, which were picked by an automatic robotic station Q-BOT and ordered into 192 384-well microtitre plates. The average insert size was 118 kb, and the library therefore represents 1.5 haploid genome equivalents of *S. otites* (whose genome size is 5.8Gb). Clones were gridded in duplicate onto Hybond N+ (Amersham, Biosciences) nitrocellulose membrane filters following a 464 pattern that allowed us to identify the position and plate number of each clone. Contamination with chloroplast DNA and mitochondrial DNA were negligible, as the nuclei had been purified by flow cytometry.

Fig. S1: Comparison of the location of the sex-linked markers in the three species of the section *Otites* with the location of their *S. latifolia* homologues (*S. latifolia* linkage groups are numbered according to Papadopulos et al., 2015; the numbers after the colons show number of markers corresponding to given linkage group).
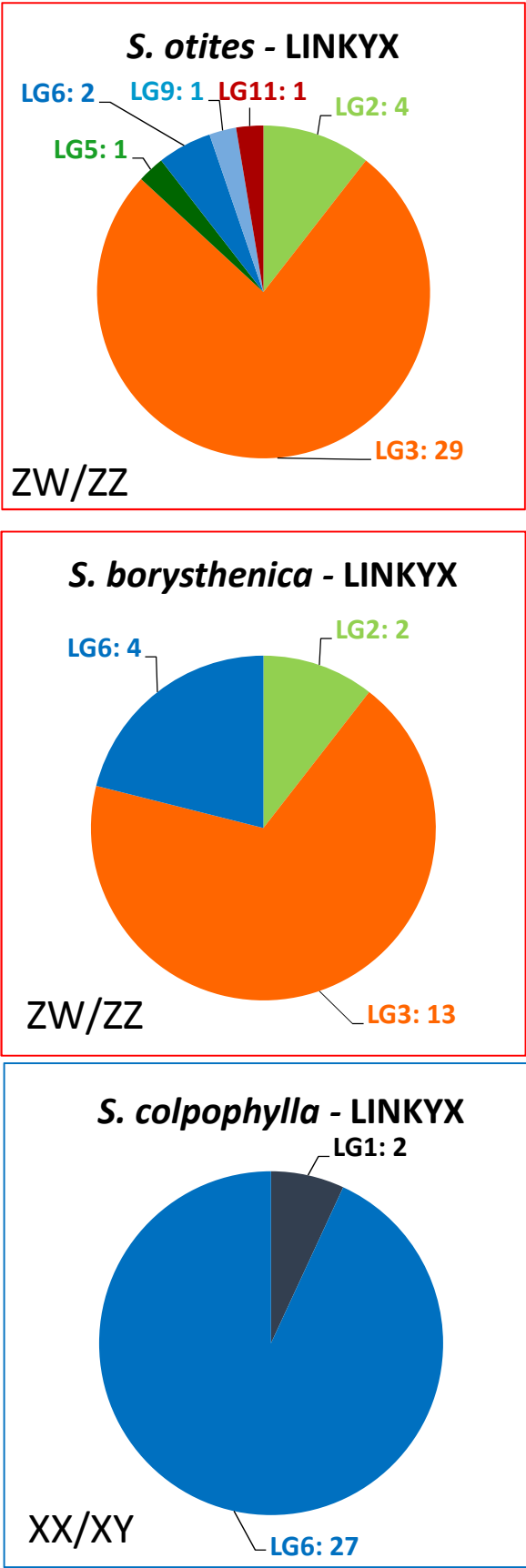
Fig. S2: Comparison of the mapping positions of the *S. latifolia* homologs of candidate sex-linked markers in S. otites and S. borysthenica. (LG3 is shown only.)

homology with
S. otites
candidate sequences

homology with
S. borysthenica
candidate sequences
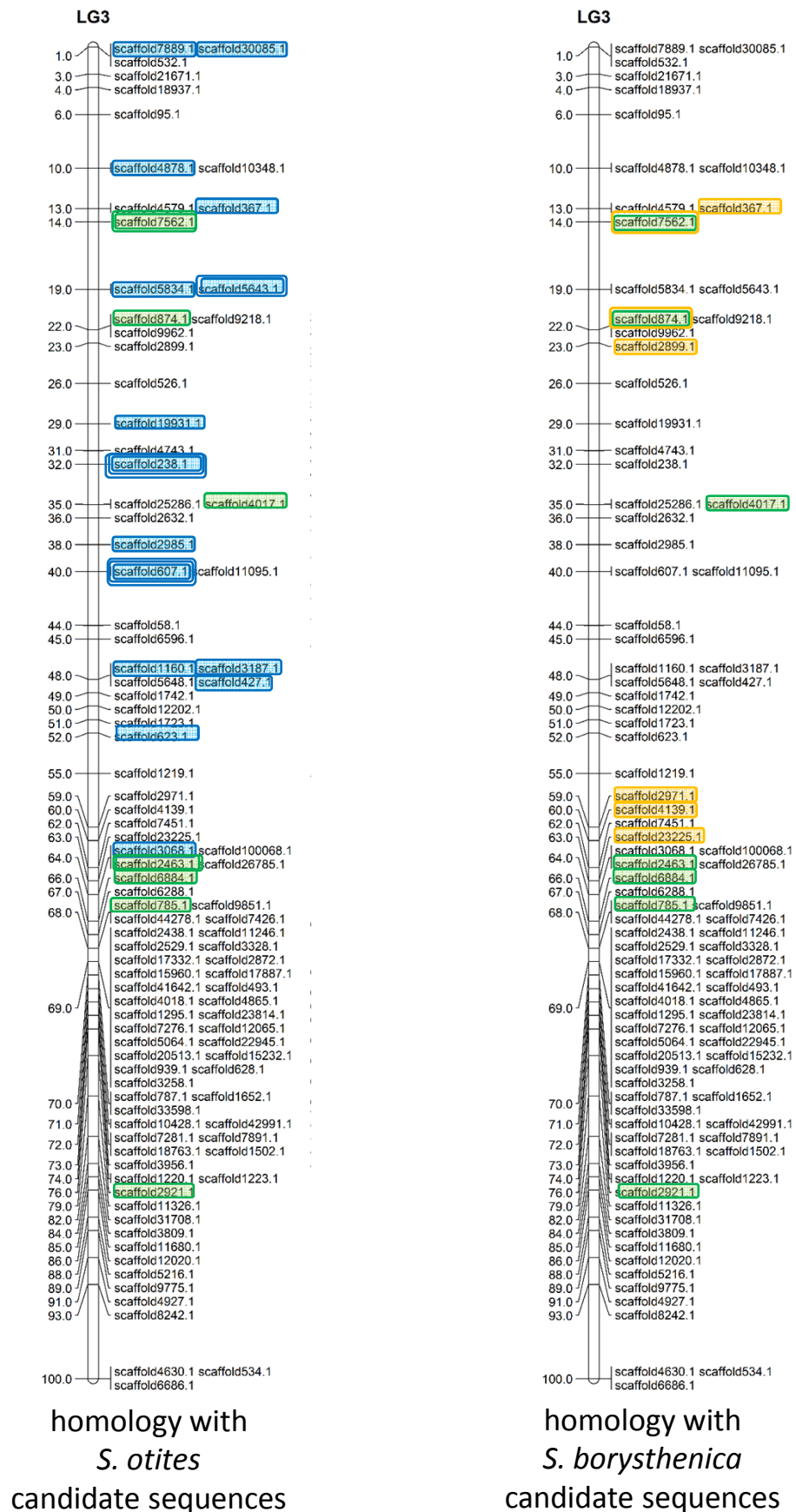
Note: The number of the marked scaffolds is lower than the number of the assigned markers as some of the scaffolds contained homologs of two or three markers (marked by double or triple line). The markers found in both species are marked in green. The markers found in *S. otites* are marked in blue. The markers found in *S. borysthenica* are marked in orange.

Fig. S3: Sexual phenotypes in *Silene sibirica*. A - hermaphrodite, B – female, C - gynomonoecious plant, D – details of the female flower in the gynomonoecious plant
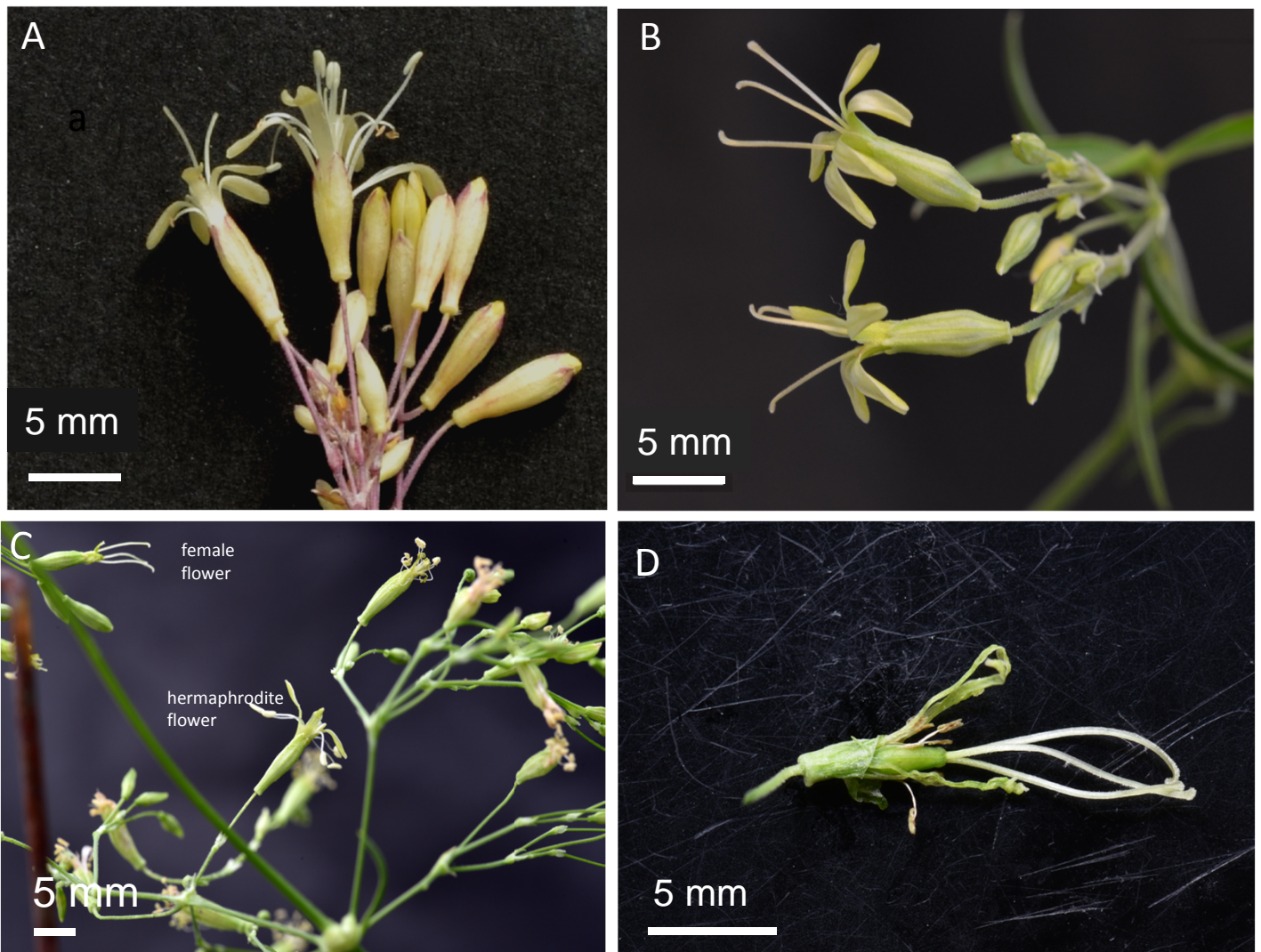
Fig. S4: Dendrograms of the Illumina RNAseq samples in *S. otites, S. borysthenica* and *S. colpophyla* based on the clustering (Euclidean distance measure, UPGMA agglomeration)

A) *S. otites* before normalization
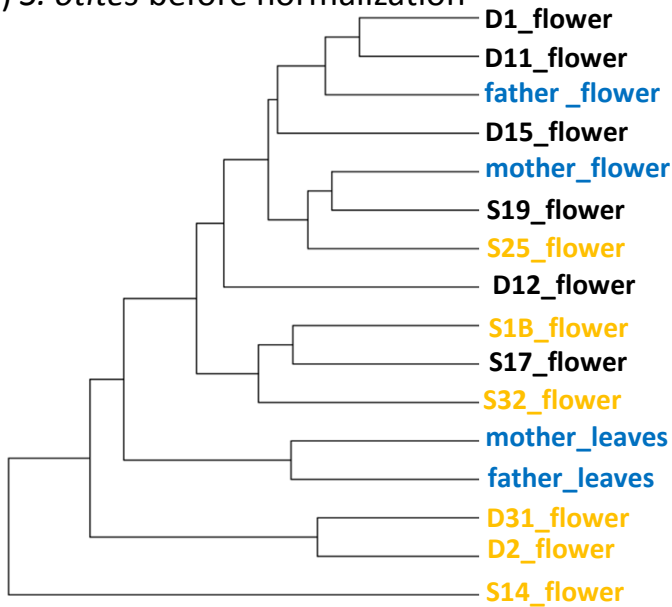
B) *S. otites* after rlog normalization
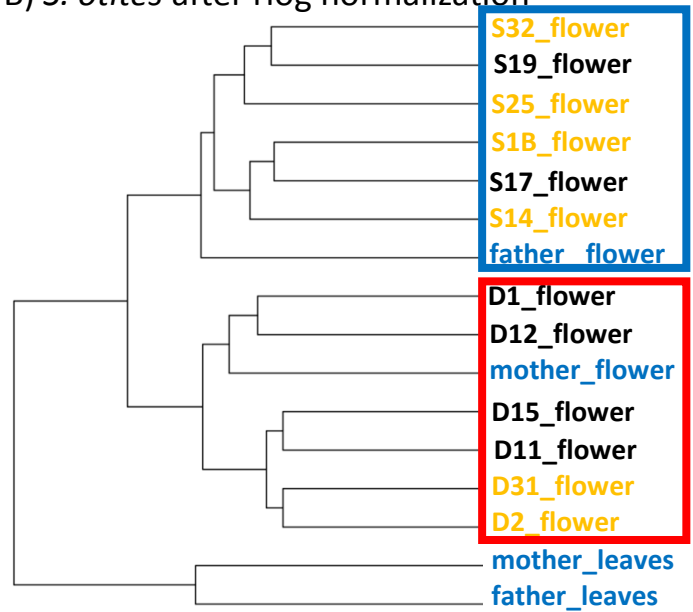
C) *S. borysthenica* before normalization
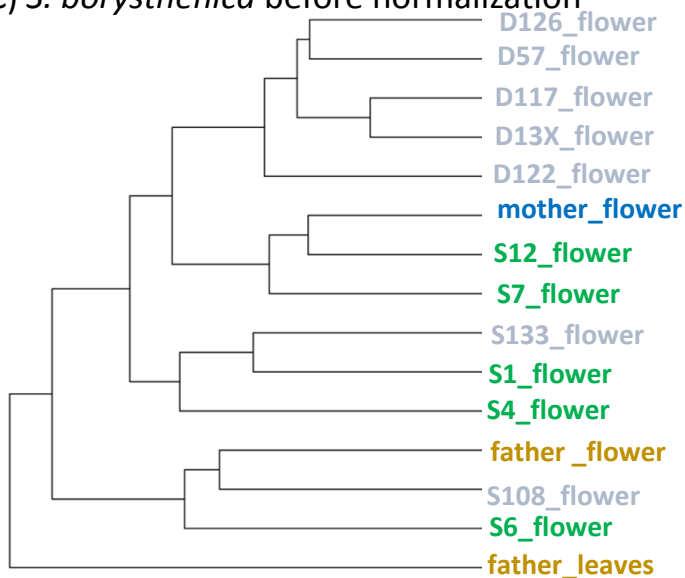
D) *S. borysthenica* after rlog normalization
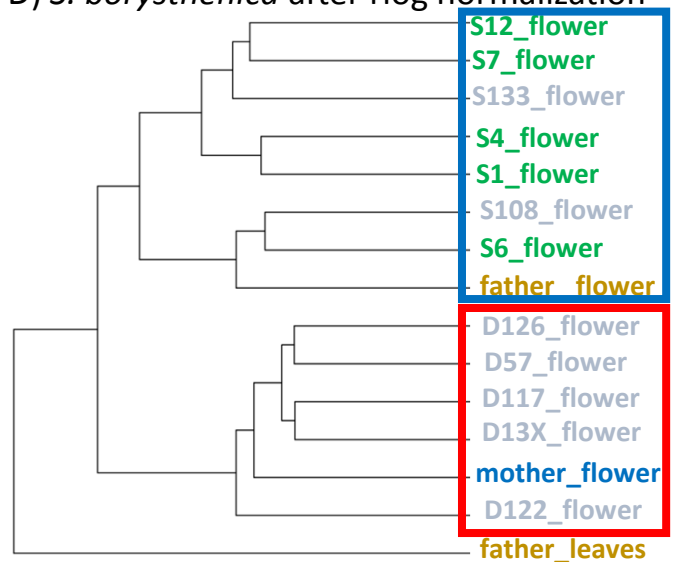
E) *S. colpophylla* before normalization
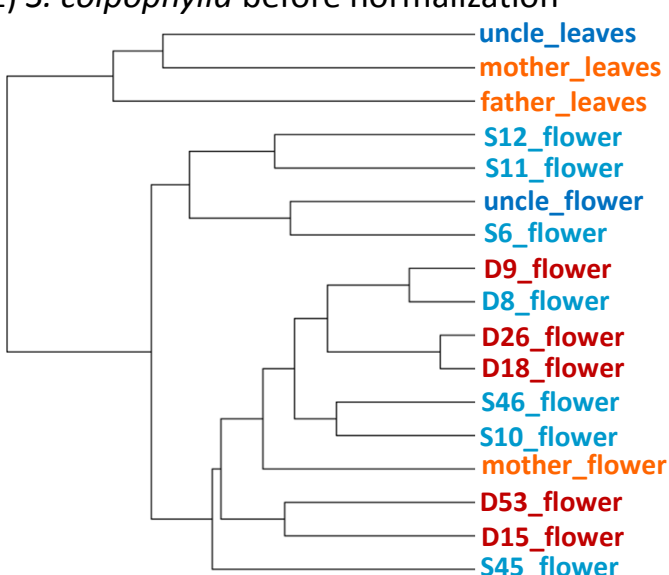
F) *S. colpophylla* after rlog normalization
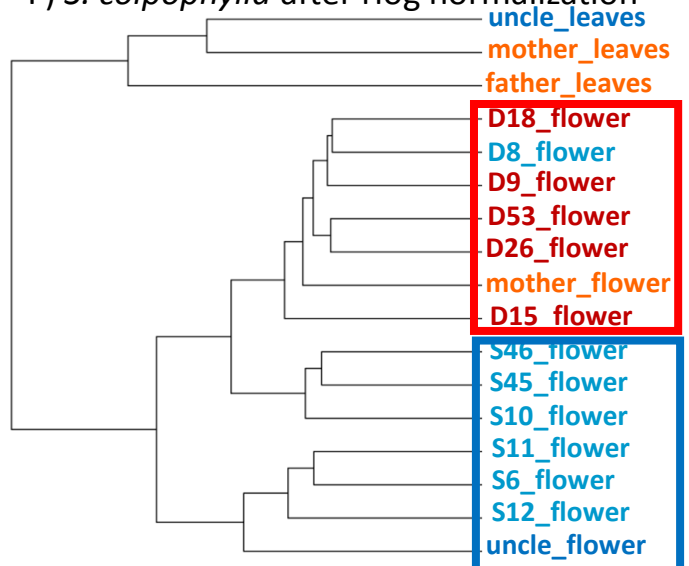
Notes: Colours indicate batch in which the sample was sequenced. D - daughter. S - son. In the analyses after normalizaton, the male flower bud samples are marked by a blue and the female flower bud samples by a red frame.

Fig. S5: Analysis of the Illumina RNAseq samples in *S. otites, S. borysthenica* and *S. colpophyla* using principal component analysis (rlog normalized counts)

A) *S. otites*: Batch effect visualisation
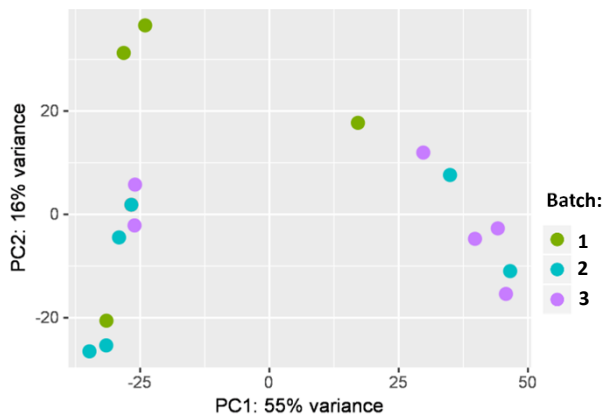
B) *S. otites*: Effects of the sample type
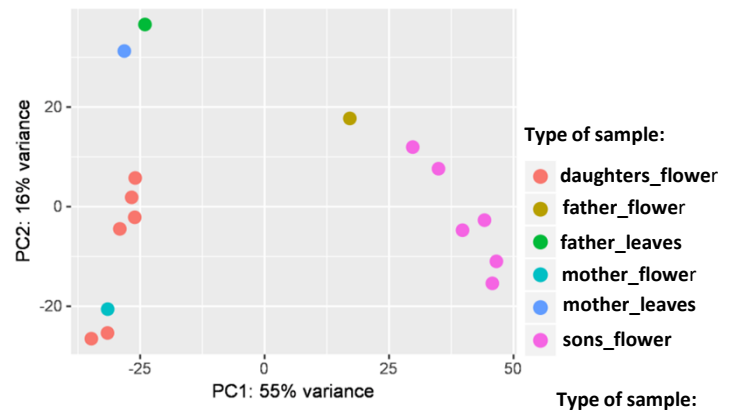
C) *S. borysthenica*: Batch effect visualisation
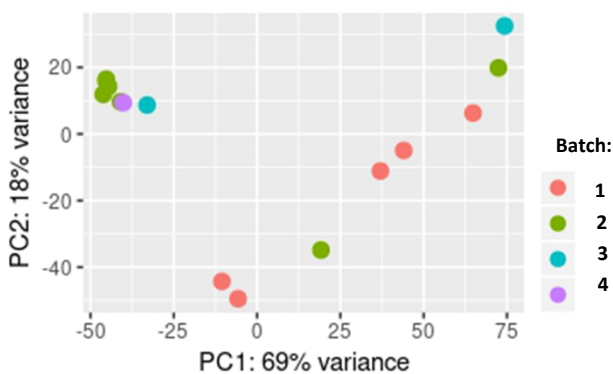
D) *S. borysthenica*: Effects of the sample type
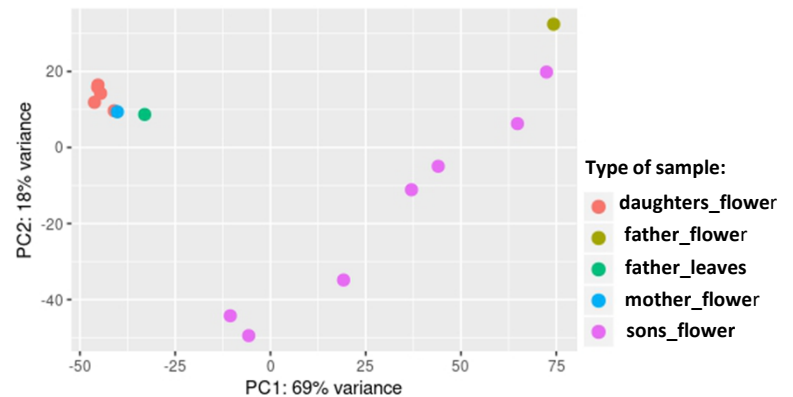
E) *S. colpophylla*: Batch effect visualisation
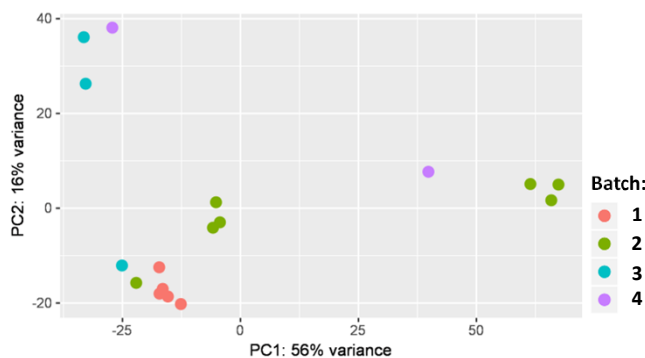
F) *S. colpophylla*: Effects of the sample type
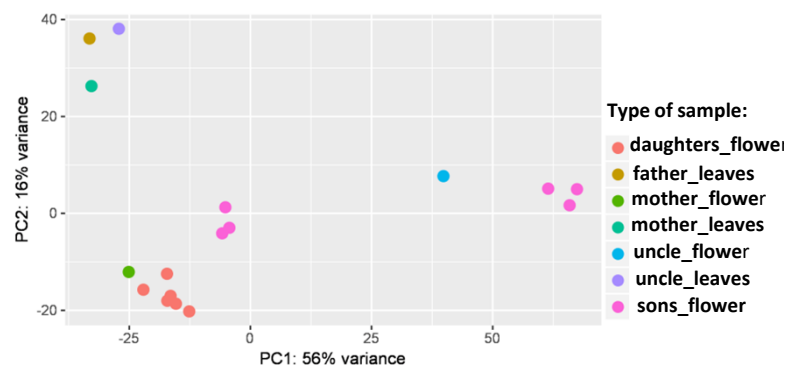
Comments: From the comparison of the graphs coloured according to batches with those coloured according to the sample types, it is apparent that the samples are clustered according to the sample type but not according to the batch in which they were sequenced. The female flower bud samples (marked as flower) show more compact clusters then male flower buds. This phenomenon is probably caused by a larger mass of quickly differentiating tissues in male flower buds. In *S. otites* and *S. colpophylla,* the male and female leaf samples (leaves) are always clustering together (in accordance with a very low number of sex specificaly expressed sequences in leaves that was found). In *S. borysthenica,* only father leaf sample was available. The LINKYX search for the sex specifically transcribed sequences was not carried out in this species.

Fig. S6: Examples of the output of the control of the sex-linkage of the candidate SNPs identified in LINKYX (both examples show *S. borysthenica* samples)

A) Analysis of SNPs in the sequence borWS_29170 using Bowtie 2 mapped reads and IGV browser.
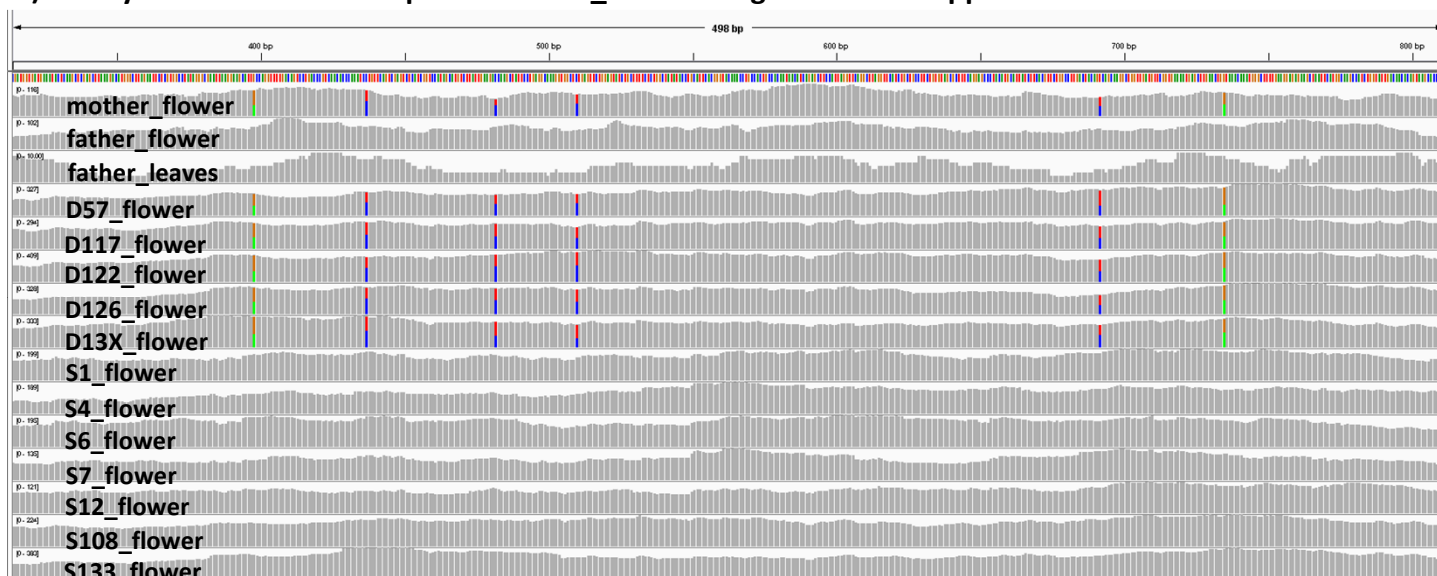
Comments: D - daughter. S - son, flower - flower buds. Figure shows 6 co-segregating W-linked polymorphisms. Heterozygous sites occur only mother and daughters.

B) Analysis of SNPs in the sequence borZS_17684 using the Bowtie 2 mapped reads and IGV browser
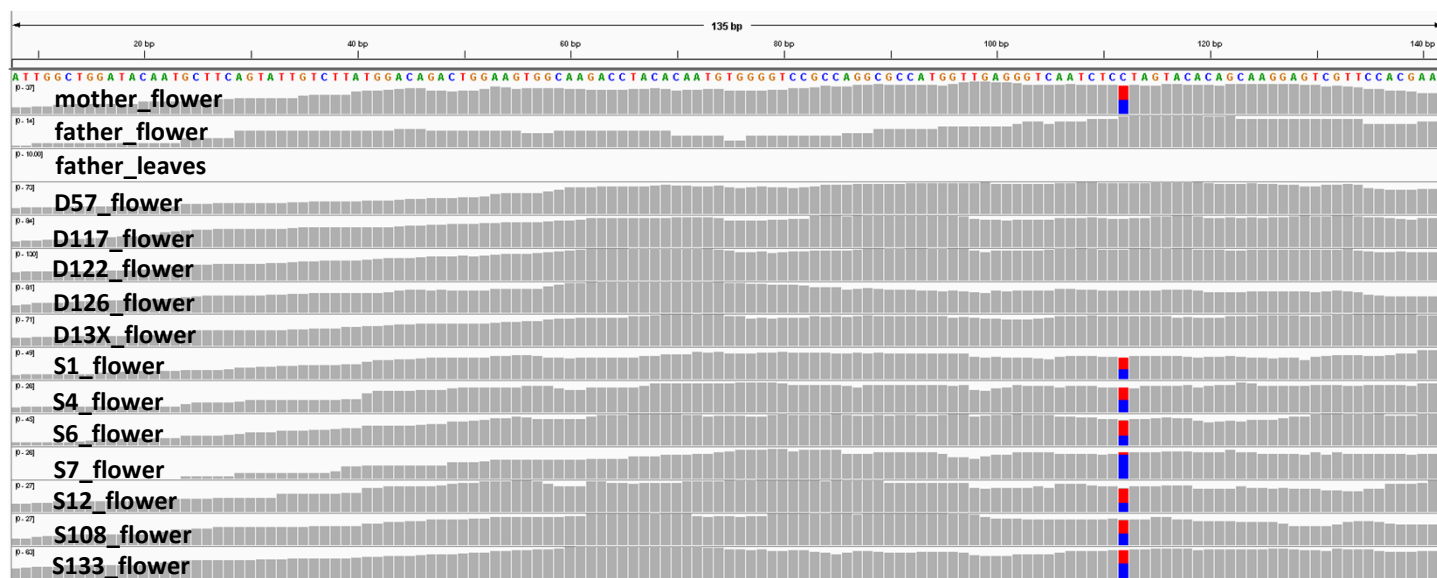
Comments: D - daughter, S - son, flower - flower buds. Figure shows one Z-linked polymorphism. Heterozygous sites occur only mother and sons. No reads present in the leaf sample obtained in father are probably caused by tissue specific expression of this sequence.

Table S1A: List of genetically mapped markers in *S. otites.*

Locus	origin of markers	homology with
otiWT43556	Illumina RNAseq marker	CRABS CLAW; zinc-finger motif containing protein
otiWT51286	Illumina RNAseq marker	leucine rich repeat receptor kinase protein
otiWT51403	Illumina RNAseq marker	tetrapeptide repeat homeobox like protein
otiWT52979	Illumina RNAseq marker	pentatricopeptide (PRR) repeat-containing protein
E224	homologues from S. latifolia	PREDICTED: 3-isopropylmalate dehydratase large subunit
E231	homologues from S. latifolia	uncharacterized protein
E233	homologues from S. latifolia	PREDICTED: alcohol dehydrogenase
E391	homologues from S. latifolia	PREDICTED: thiamine thiazole synthase 2
E514	homologues from S. latifolia	PREDICTED: peptidyl-prolyl cis-trans isomerase CYP37
E547	homologues from S. latifolia	PREDICTED: E3 ubiquitin-protein ligase SP1
E579	homologues from S. latifolia	PREDICTED: mRNA-decapping enzyme-like protein
E584	homologues from S. latifolia	uncharacterized protein
E605	homologues from S. latifolia	PREDICTED: argininosuccinate lyase
E612	homologues from S. latifolia	PREDICTED: reactive Intermediate Deaminase A
oti1390	454 sequencing marker	PREDICTED: vacuolar protein sorting-associated protein 18 homolog
oti175	454 sequencing marker	PREDICTED: chaperonin-like RbcX protein 2
oti3430	454 sequencing marker	hypothetical protein
oti388	454 sequencing marker	Pentatricopeptide repeat-containing protein
oti4124	454 sequencing marker	PREDICTED: rhodanese-like domain-containing protein 9
oti423	454 sequencing marker	uncharacterized protein
oti915	454 sequencing marker	PREDICTED: tryptophan synthase beta chain 1
otiWT52780	Illumina RNAseq marker	no homology
otiWS_10120	Illumina RNAseq marker	PREDICTED: inactive protein RESTRICTED TEV MOVEMENT 2
otiWS_18764	Illumina RNAseq marker	PREDICTED: protein SPA1-RELATED
otiWS_22526	Illumina RNAseq marker	PREDICTED: probable galacturonosyltransferase 12
otiWS_23156	Illumina RNAseq marker	uncharacterized protein
otiWS_23205	Illumina RNAseq marker	PREDICTED: HVA22-like protein f
otiWS_23758	Illumina RNAseq marker	PREDICTED: pentatricopeptide repeat-containing protein At5g18390
otiWS_25098	Illumina RNAseq marker	uncharacterized protein
otiWS_25589	Illumina RNAseq marker	no homology
otiWS_25613	Illumina RNAseq marker	PREDICTED: cell cycle checkpoint control protein RAD9A
otiWS_18439	Illumina RNAseq marker	PREDICTED: transcription factor bHLH104
otiWS_18627	Illumina RNAseq marker	uncharacterized protein
otiWS_20834	Illumina RNAseq marker	PREDICTED: RNA-binding protein 25
otiWS_2336	Illumina RNAseq marker	PREDICTED: probable glucan 1,3-alpha-glucosidase
otiWS_26046	Illumina RNAseq marker	RNase_T domain-containing protein
otiWS_26246	Illumina RNAseq marker	PREDICTED: F-actin-capping protein subunit alpha
otiWS_7824	Illumina RNAseq marker	uncharacterized protein

Note: The completely sex linked sequences are in bold.
Labelling of markers:
OtiWT- markers found based on their female specific transcription (detected using LINKYX in Illumina RNAseq data). All four completely sex linked markers in the map show also female specific PCR amplification.
OtiWS - markers found based on the W-linked SNP-polymorphism (detected using LINKYX in Illumina RNAseq data).
oti - markers found based on 454 RNAseq data.
E – markers found based on the homology of the ZW chromosome pair with the linkage group 6 in *S. latifolia* in the numbering of Bergero *et al*. (2013) (LG3 in numbering according to Papadopulos et al., 2015). Each this marker corresponds to the homologue in *S. latifolia* with the same label.

Table S1B: List of genetically mapped markers in *S. colpophylla.*

Locus	origin of markers	homology with
colpXS_16839	Illumina RNAseq marker	PREDICTED: U-box domain-containing protein 44
colpYS_10497	Illumina RNAseq marker	PREDICTED: protein trichome berefringence-like 7
colpYS_12469	Illumina RNAseq marker	Complex 1 LYR protein
colpYS_14201	Illumina RNAseq marker	PREDICTED: probable arabinosyltransferase ARAD1
colpYS_15390	Illumina RNAseq marker	uncharacterized protein
colpYS_17495	Illumina RNAseq marker	no homology
colpYS_18199	Illumina RNAseq marker	PREDICTED: protein trichome berefringence-like 7
colpYS_18468	Illumina RNAseq marker	PREDICTED: phosphatidylinositol 3- and 4-kinase family protein
colpYS_18982	Illumina RNAseq marker	PREDICTED: filament-like plant protein
colpYT_19195	Illumina RNAseq marker	no homology
colpYS_24813	Illumina RNAseq marker	transcription factor bHLH
colpYS_25642	Illumina RNAseq marker	CLV1-like LRR receptor kinase
colpYS_4053	Illumina RNAseq marker	PREDICTED: TLC domain-containing protein 2
colpYS_4711	Illumina RNAseq marker	no homology
colpYT_50424	Illumina RNAseq marker	no homology
colpYS_7668	Illumina RNAseq marker	CLV1-like LRR receptor kinase
colpYS_8617	Illumina RNAseq marker	PREDICTED: protein ROOT PRIMORDIUM DEFECTIVE 1
colpYS_8940	Illumina RNAseq marker	hypothetical protein
E265	homologue from *S. latifolia* (Qiu et al. 2010)	FCLY (farnesylcysteine lyase) or prenylcysteine oxidase
oti193	454 sequencing marker from S. otites	RNA-binding S4 domain-containing protein
colpXS_20384	Illumina RNAseq marker	no homology
colpXS_20972	Illumina RNAseq marker	PREDICTED: NAC domain-containing protein 72-like
colpXS_10827	Illumina RNAseq marker	PREDICTED: sulfate transporter 1.2 isoform X1
colpXS_13981	Illumina RNAseq marker	PREDICTED: transcription factor TGA3
colpXS_14571	Illumina RNAseq marker	hypothetical protein
colpXS_15384	Illumina RNAseq marker	PREDICTED: probable mitochondrial adenine nucleotide transporter BTL3 isoform X1
colpXS_17026	Illumina RNAseq marker	PREDICTED: probable tRNA modification GTPase MnmE
colpXS_18134	Illumina RNAseq marker	PREDICTED: Niemann-Pick C1 protein isoform X3
colpXS_19384	Illumina RNAseq marker	PREDICTED: 3-ketodihydrosphingosine reductase-like
colpXS_19736	Illumina RNAseq marker	PREDICTED: branched-chain-amino-acid aminotransferase 2, chloroplastic isoform X2
colpYS_13383	Illumina RNAseq marker	PREDICTED: retinoblastoma-related protein isoform X1
colpYS_14000	Illumina RNAseq marker	PREDICTED: F-box protein At4g00755
colpYS_14076	Illumina RNAseq marker	no homology
colpYS_14647	Illumina RNAseq marker	PREDICTED: probable alkaline/neutral invertase B
colpYS_15303	Illumina RNAseq marker	PREDICTED: flap endonuclease GEN-like 1
colpYS_16496	Illumina RNAseq marker	PREDICTED: biogenesis of lysosome-related organelles complex 1 subunit 1
colpYS_16597	Illumina RNAseq marker	calcium-dependent protein kinase 16
colpYS_17650	Illumina RNAseq marker	PREDICTED: histone acetyltransferase of the MYST family 1
colpYS_18193	Illumina RNAseq marker	hypothetical protein
colpYS_19627	Illumina RNAseq marker	PREDICTED: calcium-dependent protein kinase 11
colpYS_19951	Illumina RNAseq marker	PREDICTED: serine/threonine-protein phosphatase 7 long form homolog
colpYS_20903	Illumina RNAseq marker	PREDICTED: serine/arginine-rich splicing factor RS31-like isoform X2
colpYS_20980	Illumina RNAseq marker	NAC2
colpYS_21702	Illumina RNAseq marker	xylulose kinase
colpYS_2485	Illumina RNAseq marker	PREDICTED: hydroquinone glucosyltransferase
colpYS_25807	Illumina RNAseq marker	PREDICTED: cullin-1 isoform X2

Note: The sequences showing complete sex linkage in the mapping population are in bold.
Labelling of the markers:
colpYT- markers found based on their male specific transcription (detected using LINKYX in Illumina RNAseq data). Two completely sex linked markers from this category show also male specific PCR amplification from genomic DNA.
colpYS - markers found based on the Y-linked SNP-polymorphism (detected using LINKYX in Illumina RNAseq data).
colpXS - markers found based on the X-linked SNP-polymorphism (detected using LINKYX in Illumina RNAseq data).
E265 – marker found based on the homology of the XY chromosome pair with the linkage group 6 in *S. latifolia* in numbering according to Papadopulos et al., 2015). This marker corresponds to the homologue E265 in *S. latifolia* (Qiu et al. 2010).

Table S2: Analysis of the linkage disequilibrium at sexual phenotype and four tested loci.

population	sex linked locus	results of the the tests for the presence of the presumed W-linked alleles				
		females			males	
		positive	negative		positive	negative
Kralovo Pole	otiW43556	12	0		0	18
	otiW51286	12	0		0	18
	otiW51403	12	0		0	18
	otiW52979	12	0		0	18
Rohatec	otiW43556	15	0		0	17
	otiW51286	15	0		0	17
	otiW51403	15	0		0	17
	otiW52979	15	0		0	17

Table S3: Overview of the analysis of the completely sex-linked sequences and their surroundings in *S. otites*.

As the order of the markers was unknown, the minimal size of the non-recombining region was calculated as the sum of the size of two smalest BACs plus the sum of the smallest distances from the contig ends in the two remaining BACs. According to this approach, the minimal size of the non-recombining region in S. otites is more than 180 kb.

fully sex-linked gene	homology	number of BACs	Homologs of other genes in BACs	BAC size (kb)	smaller distance between the marker and the BAC end (kb)	larger distance between the marker and the BAC end (kb)
otiWT_51286	leucine rich repeat receptor kinase protein	4	no other gene present	120.4	25.2	27.5
otiWT_52979	pentatricopeptide (PPR) repeat-containing protein	2	wall associated kinase	more than 49.5*	21.4	27.9
otiWT_43556	CRABS CLAW; zinc-finger motif containing protein;	1	no other gene present	93	26.2	47.9
otiWT_51403	tetrapeptide repeat homeobox like protein	3	no other gene present	99.1	12.6	38.7

* As the complete assembly was not possible in the BAC carrying otiW52979, its minimal length is given as the lenth of the largest contig.

Table S4A: Analysis of combinations of the ancestral states of sex determining system in the most recent common ancestors of the section Otites and in the MRCA of the group Otites in BayesTraits (S. pseudotites set as Y). First letter corresponds to ancestral state in the MRCA of the section Otites and the second to the ancestral state in the MCA of the group Otites (W-female heterogamety, N-non-dioecy, Y-male heterogamety).

Note: * = positive evidence: 2 < 2×difference in ln(marginal likelihood) > 6, ** = strong evidence: 6 < 2×difference in ln(marginal likelihood) > 10, *** = very strong evidence: 10 < 2×difference in ln(marginal likelihood) (Kass & Raftery 1995)

Combinations of the ancestral states	Ln (marginal likelihood)	2×differences in ln(marginal likelihood)								
		-WW	-YY	-WY	-NW	-NY	-YW	-NN	-WN	-YN
WW	-10.388936	0.000	3.255*	5.279*	5.626*	6.763**	9.025**	9.269**	14.867***	19.810***
YY	-12.016426	-3.255	0.000	2.024*	2.371*	3.508*	5.770*	6.014**	11.612***	16.555***
WY	-13.028208	-5.279	-2.024	0.000	0.347	1.484	3.746*	3.990*	9.589**	14.532***
NW	-13.201756	-5.626	-2.371	-0.347	0.000	1.137	3.399*	3.642*	9.242**	14.185***
NY	-13.770253	-6.763	-3.508	-1.484	-1.137	0.000	2.262*	2.506*	8.105**	13.048***
YW	-14.901343	-9.025	-5.770	-3.746	-3.399	-2.262	0.000	0.244	5.842*	10.786***
NN	-15.023221	-9.269	-6.014	-3.990	-3.643	-2.506	-0.244	0.000	5.599*	10.542***
WN	-17.822585	-14.867	-11.612	-9.589	-9.242	-8.105	-5.842	-5.599	0.000	4.943*
YN	-20.294095	-19.810	-16.555	-14.532	-14.185	-13.048	-10.786	-10.542	-4.943	0.000

Table 4B: Analysis of combinations of the ancestral states of sex determining system in the most recent common ancestors of the section Otites and in the MRCA of the group Otites in BayesTraits (S. pseudotites set as WY). First letter corresponds to ancestral state in the MRCA of the section Otites and the second to the ancestral state in the MCA of the group Otites (W-female heterogamety, N-non-dioecy, Y-male heterogamety).

Note: * = positive evidence: 2 < 2×difference in ln(marginal likelihood) > 6, ** = strong evidence: 6 < 2*difference in ln(marginal likelihood) > 10, *** = very strong evidence: 10 < 2*difference in ln(marginal likelihood) (Kass & Raftery 1995)

Combinations of the ancestral states	Ln (marginal likelihood)	2×differences in ln(marginal likelihood)								
		-WW	-NW	-YY	-YW	-WY	-NN	-NY	-WN	-YN
WW	-7.602	0.000	5.665*	8.220**	9.260**	10.341***	11.733***	11.787***	17.337***	22.811***
NW	-10.434	-5.665	0.000	2.555*	3.595*	4.676*	6.068**	6.122**	11.672***	17.145***
YY	-11.712	-8.220	-2.555	0.000	1.040	2.121*	3.513*	3.567*	9.117**	14.590***
YW	-12.232	-9.260	-3.595	-1.040	0.000	1.081	2.473*	2.527*	8.077**	13.550***
WY	-12.772	-10.341	-4.676	-2.121	-1.081	0.000	1.392	1.446	6.996**	12.469***
NN	-13.468	-11.733	-6.068	-3.513	-2.473	-1.392	0.000	0.054	5.604*	11.077***
NY	-13.495	-11.788	-6.122	-3.567	-2.527	-1.446	-0.054	0.000	5.550*	11.023***
WN	-16.270	-17.337	-11.672	-9.117	-8.077	-6.996	-5.604	-5.550	0.000	5.473*
YN	-19.007	-22.811	-17.145	-14.590	-13.550	-12.469	-11.077	-11.023	-5.473	0.000

Table S5: Overview of the plant species and samples used in analyses.

Species	voucher or collector	Geographic origin	RNAseq origin
Silene baschkirorum Janisch.	BRNU 590993	Muradym (previous name Yuldybaevo 3-e village; Mrakovo distr., Bashkortostan Republic, Russian Federation)	this manuscript
Silene borysthenica (Gruner) Walters	BRNU 576274	eastern periphery of Abakan City (Khakasia, Minusinsk Basin; Russian Federation)	this manuscript
Silene colpophylla Wrigley	Janousek IBPBR 27	originally cultivated at ENS Lyon, Lyon, France	this manuscript
Silene nocturna L.	BRNU615725	Kalyviani (distr. Chaniá Gramvousa Peninsula, Crete)	this manuscript
Silene nutans L.	Bengt Oxelman, University of Gothenburgh, Sweden		this manuscript
Silene otites Sm.	Janousek IBPBR 1	Nantes (Loire-Atlantique region, France)	this manuscript
Silene paradoxa L.	Deborah Triant, University of Virginia, USA	cultivated at university of Virginia, USA	publically available, BioSample: SAMN02351094;
Silene pseudotites Besser ex Reichenb.	Janousek IBPBR 55	Basovizza (municipality of Trieste, Province of Trieste, Italy)	this manuscript
Silene sibirica (L.) Pers.	BRNU631315	Krasnogorka village (Omsk region, Poltavka district, Russian Federation)	this manuscript
Silene wolgensis Otth	BRNU 590996	Novoaleksandrovka village (18 km SW of Zilair) (Bashkortostan Republic, Zilairskoe plateau, Zilair distr.; Russian Federation)	this manuscript

Table S6:   List of the accession numbers of the NCBI BioProjects storing the RNAseq data.

Species	TaxId	BioProject ID	type of data
Silene baschkirorum	1390714	PRJNA407407	Transcriptome Illumina
Silene borysthenica	1390715	PRJNA404055	Transcriptome Illumina
Silene colpophylla	512022	PRJNA361444	Transcriptome Illumina
Silene otites	42039	PRJNA403783	Transcriptome 454
Silene otites	42039	PRJNA401641	Transcriptome Illumina
Silene pseudotites	42041	PRJNA407280	Transcriptome Illumina
Silene sibirica	1291645	PRJNA407314	Transcriptome Illumina
Silene wolgensis	1389442	PRJNA407297	Transcriptome Illumina

Table S7:   Overview of the Illumina RNAseq sequenced samples studied by LINKYX

Silene otites

type of specimen	sample_ID	lane_ID	raw reads (left+right)	trimmed reads (paired, left + right)	reference: father flower buds			reference: mother + daugthers (flower buds)		
					mapped	unmapped	% mapped	mapped	unmapped	% mapped
mother-leaves	NNN18L	OSR13s005080	70954976	60475858	55884502	4591356	92%	57454103	3021755	95%
mother-flower buds	NNN18P	OSR13s005080	63497510	53753330	49164565	4588765	91%	50595473	3157857	94%
father-leaves	NNN27L	OSR13s005080	63351950	53240266	49471205	3769061	93%	50193590	3046676	94%
father-flower buds	NNN27P	OSR13s005080	73789112	62611828	58666877	3944951	94%	57799122	4812706	92%
daughters-flower buds	NNN18x27_11FP	OSR14s007369	50281150	42796220	40311011	2485209	94%	40628257	2167963	95%
	NNN18x27_12FP	OSR14s007369	76914222	67953504	62359183	5594321	92%	64241110	3712394	95%
	NNN18x27_15FP	OSR14s007369	82893462	73432536	68794151	4638385	94%	69625097	3807439	95%
	NNN18x27_1FP	OSR14s007369	50704846	46292274	43570561	2721713	94%	44088454	2203820	95%
	NNN18x27_2FP	OSR15s008719	114110562	108218530	100418719	7799811	93%	101745376	6473154	94%
	NNN18x27_31FP	OSR15s008719	116844498	109860928	100575169	9285759	92%	102626178	7234750	93%
sons-flower buds	NNN18x27_14MP	OSR15s008719	121084668	114160060	102000059	12160001	89%	104747391	9412669	92%
	NNN18x27_17MP	OSR14s007369	99980922	77105218	70444017	6661201	91%	71303848	5801370	92%
	NNN18x27_19MP	OSR14s007369	59486732	52122456	48561166	3561290	93%	48395139	3727317	93%
	NNN18x27_1BMP	OSR15s008719	84209270	79267464	72909619	6357845	92%	73570393	5697071	93%
	NNN18x27_25MP	OSR15s008719	74850408	70653560	65092312	5561248	92%	65243526	5410034	92%
	NNN18x27_32MP	OSR15s008719	88160726	83586110	76393882	7192228	91%	76792548	6793562	92%

Silene borysthenica

type of specimen	sample_ID	lane_ID	raw reads (left+right)	trimmed reads (paired, left + right)	reference: father flower buds			reference: mother + daugthers (flower buds)		
					mapped	unmapped	% mapped	mapped	unmapped	% mapped
mother-flower buds	BOR_5FP	OSR13s005080	70986938	60535752	51405283	9130469	85%	57394158	3141594	95%
father-leaves	NEW_BOR_3L	OSR14s007368	46870138	34770910	31521559	3249351	91%	32654613	2116297	94%
father-flower buds	NEW_BOR_3P	OSR14s007368	75799902	64518596	60130677	4387919	93%	56852092	7666504	88%
daughters-flower buds	BB_57FP	OSR16s010836	142012050	112572718	100018589	12554129	89%	107266440	5306278	95%
	BB_117FP	OSR16s010836	129036354	101173034	91547167	9625867	90%	96121690	5051344	95%
	BB_122FP	OSR16s010836	126438494	100712834	92183499	8529335	92%	96217456	4495378	96%
	BB_126FP	OSR16s010836	112921298	87638264	79320530	8317734	91%	83359904	4278360	95%
	BB_13XFP	OSR16s010836	132560834	104558974	95962601	8596373	92%	99992934	4566040	96%
sons-flower buds	BB_1MP	OSR15s008724	91021474	85778596	74860355	10918241	87%	77863602	7914994	91%
	BB_4MP	OSR15s008724	122179108	114319088	99774242	14544846	87%	103992581	10326507	91%
	BB_6MP	OSR15s008724	101449614	93813624	84592968	9220656	90%	83182051	10631573	89%
	BB_7MP	OSR15s008724	87475176	82419008	72895390	9523618	88%	74286784	8132224	90%
	BB_12MP	OSR15s008724	62164452	58309236	51627754	6681482	89%	51984175	6325061	89%
	BB_108MP	OSR16s010836	103852006	80710546	73588918	7121628	91%	73147941	7562605	91%
	BB_133MP	OSR16s010836	125729588	94446450	84236253	10210197	89%	86462796	7983654	92%

Silene colpophylla

type of specimen	sample_ID	lane_ID	raw reads (left+right)	trimmed reads (paired, left + right)	reference: mother flower buds			reference: father (leaves) + sons (flower buds)		
					mapped	unmapped	% mapped	mapped	unmapped	% mapped
mother-leaves	AL	OSR14s007366	81365452	71020128	68219559	2800569	96%	65094996	5925132	92%
mother-flower buds	AP	OSR14s007366	70002736	58695892	55589384	3106508	95%	54711208	3984684	93%
father-leaves	BL	OSR14s007366	59077754	49428900	21258152	28170748	43%	22997607	26431293	47%
uncle-leaves	A1_L	OSR13s005080	62309456	62606496	56616439	6016439	90%	58832677	3773819	94%
uncle-flower buds	A1_P	OSR13s005080	82168286	69900880	59609438	10291442	85%	65008062	4892818	93%
daughters-flower buds	AxB_8P	OSR14s007367	53680064	47620884	44313713	3307171	93%	44359826	3261058	93%
	AxB_9P	OSR14s007368	59559668	49369106	46082392	3286714	93%	46182804	3186302	94%
	AxB_15P	OSR14s007368	73420962	59894500	55775767	4118733	93%	55802174	4092326	93%
	AxB_18P	OSR14s007368	36737780	29862268	27673614	2188654	93%	27910452	1951816	93%
	AxB_26P	OSR14s007368	37950966	30356706	28011562	2345144	92%	28241805	2114901	93%
	AxB_53P	OSR14s007368	83923538	69234046	64033971	5200075	92%	64311453	4922593	93%
sons-flower buds	AxB_6P	OSR14s007367	67003158	59874552	51216116	8658436	86%	56401754	3472798	94%
	AxB_10P	OSR14s007367	48473938	43502350	38736084	4766266	89%	40496151	3006199	93%
	AxB_11P	OSR14s007367	45500730	38189340	32497723	5691617	85%	35701260	2488080	93%
	AxB_12P	OSR14s007367	64401312	58337356	47266603	11070703	81%	54572511	3764845	94%
	AxB_45P	OSR14s007367	69519626	62606496	55847056	6759440	89%	58554164	4052332	94%
	AxB_46P	OSR14s007367	55960836	50045002	44356247	5688755	89%	46888865	3156137	94%